Montana 2010 Avian Influenza Surveillance Project Report

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The emergence and spread of the highly pathogenic avian influenza (AI) H5N1-Asian strain (HP-H5N1) in Asia, the Middle East, Europe, and Africa has elevated concern about potential expansion of the disease to North America. Such an event could have negative affects on the poultry industry, humans, and wild bird populations (World Health Organization 2007). The role of wild migratory birds in the movement and transmission of HP-H5N1 is poorly understood and strongly contested (Krauss *et al.* 2007, Peterson *et al.* 2007, van Gils *et al.* 2007). Circumstantial evidence suggests wild waterfowl may introduce AI viruses in the low pathogenic form to poultry flocks (World Health Organization 2007) and some species of waterfowl may asymptomatically carry HP-H5N1 to new geographical areas during long distance migration (Chen *et al.* 2006, Lvov *et al.* 2006, Al-Azemi *et al.* 2008, but see Weber and Stilianakis 2007). Molting, migration stopovers, and wintering grounds allow birds to exist in high densities and provide opportunities for the transmission of low pathogenic avian influenza (LPAI) viruses between species, and wild and captive birds (Olsen *et al.* 2006, Chen and Holmes 2009), which then may recombine or mutate into a highly pathogenic form (Scholtissek *et al.* 1978, Ungchusak *et al.* 2005, Dugan *et al.* 2008).

The U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services (WS) and the U.S. Fish and Wildlife Service (USFWS) initiated and funded a nationwide avian influenza surveillance project for the early detection of HP-H5N1 in 2006, which continued annually through 2010. The surveillance included all four flyways, all states, and tribal lands in the United States. Montana was considered a top priority state because the Pacific and Central Flyways divide the state and it borders Canada. Montana Fish, Wildlife and Parks (FWP), WS, and USFWS conducted sample collections for the 2010 Montana AI surveillance project. The Montana Department of Livestock Diagnostic Laboratory (MDoL), National Veterinary Services Laboratory (NVSL), and the U.S. Geological Survey National Wildlife Health Center (NWHC) tested samples. The Tribal Nations and the Department of Public Health and Human Services were also collaborators. The objective of the project was to employ multiple sampling strategies to maximize the chance of detecting HP-H5N1, including sampling live and hunter-harvested waterfowl, conducting state-wide systematic mortality/morbidity transects, and collecting samples from wild bird mortality/morbidity events.

Sample Design

The Montana AI surveillance sampling strategy was an adaptive step-down approach from the U.S. Interagency Strategic Plan (Interagency Asian HPAI Early Detection Working Group 2006) and the Pacific and Central Flyway plans (Pacific Flyway Council 2006, Central Flyway Council 2006). The above plans suggested that $\geq$200 samples would be required to detect one positive HP-H5N1 sample in a defined bird population of >1000 individuals with a 95% confidence interval at a disease prevalence of $\leq$1.5%.

Swab Sampling Surveillance

The criteria outlined in the 2006 Montana Sampling Plan (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2006) stated that FWP and WS would collaboratively collect swab samples from live and hunter-harvested birds from identified species of concern. Methods used in 2006 included collecting only a cloacal swab sample from each bird; in subsequent years of surveillance an additional oropharyngeal swab was collected and placed in the same vial with a cloacal swab to amplify the sample (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2007). Laboratory testing of AI samples in 2006

included combining up to five individual cloacal samples in a sample pool to initially screen for all influenza A viruses. The protocol for the screening of samples in 2007 changed to testing each swab sample individually rather than pooling samples. Target sample numbers varied across years to adjust for the increased testing costs associated with initial screening (2006: n=2000, 2007: n=1500, 2008: n=1600, 2009: n=1400). The original 2010 sampling goal was to obtain a total of 1400 cloacal-oropharyngeal samples in Montana, 600 of which were to be collected by FWP and 800 by WS (USDA-APHIS-Wildlife Services, U.S. States and Tribes 2010). However, because funding for WS diagnostic services was redirected on 1 October, WS necessarily halted swab collection at the end of September. FWP was able to continue sample collection throughout the 2010 sampling season which ended in November. As a result, overall swab collection was reduced to approximately 800 during 2010. Cloacal and oropharyngeal sampling strategies were to: 1) coordinate with USFWS National Wildlife Refuge waterfowl trapping and banding operations, 2) sample hunter-harvested waterfowl at National Wildlife Refuges and on state-owned lands, and 3) trap wild and semi-domestic waterfowl on urban ponds (Figure 1).

Mortality/morbidity Surveillance

Mortality/morbidity samples were collected statewide by FWP in collaboration with USFWS throughout each year of the project. Prospective mortality/morbidity surveillance was added in 2007 as an AI detection method to systematically survey species of concern across Montana (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2007). Mortality/morbidity surveillance began in summer during 2007, 2008, and 2010, while additional spring surveillance was conducted in 2009 to capture the shorebird migration. Weekly surveys were conducted concurrently at $\geq$ 6 sites throughout the state on bodies of water that supported species capable of demonstrating clinical symptoms due to highly pathogenic infection (U.S. Department of the Interior Fish and Wildlife Service 2008).

METHODS

Cloacal and Oropharyngeal Sampling

The cloacal and oropharyngeal sample design assumed: 1) the populations of birds to be sampled were homogeneous and accessible, 2) HP-H5N1 was uniformly distributed across bird populations, and 3) representative sampling would be random and unbiased. Because these assumptions could not be met for wild migratory waterfowl, sample sizes were increased and sampling was extrapolated across large landscapes for multi-state and flyway sampling efforts in an attempt to account for biases (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2006). Cloacal and oropharyngeal sampling was spatially distributed across Montana and temporally distributed from July through November. The Implementation Plan for HPAI Surveillance in Wild Migratory Birds in the United States (USDA-APHIS-Wildlife Services, U.S. States and Tribes 2010) called for 30% of the swab samples to be collected from resident or non-migrating waterfowl and the remaining 70% to be collected from migratory species upon arrival in fall through freeze-up. However, redirection of funding for WS wild bird sampling on 1 October 2010 shifted the sample design. The change resulted in an increase of resident and non-migrating bird samples to 52%, a decrease of migrating bird samples during the hunter-harvest period to 48%, and an overall decrease in swab samples to 60% of the original goal for 2010 (Table 1). Waterfowl species identified as potential carriers of HP-H5N1 but not expected to exhibit clinical disease were targeted for surveillance. Species of primary concern for the 2010 live and hunter-harvested

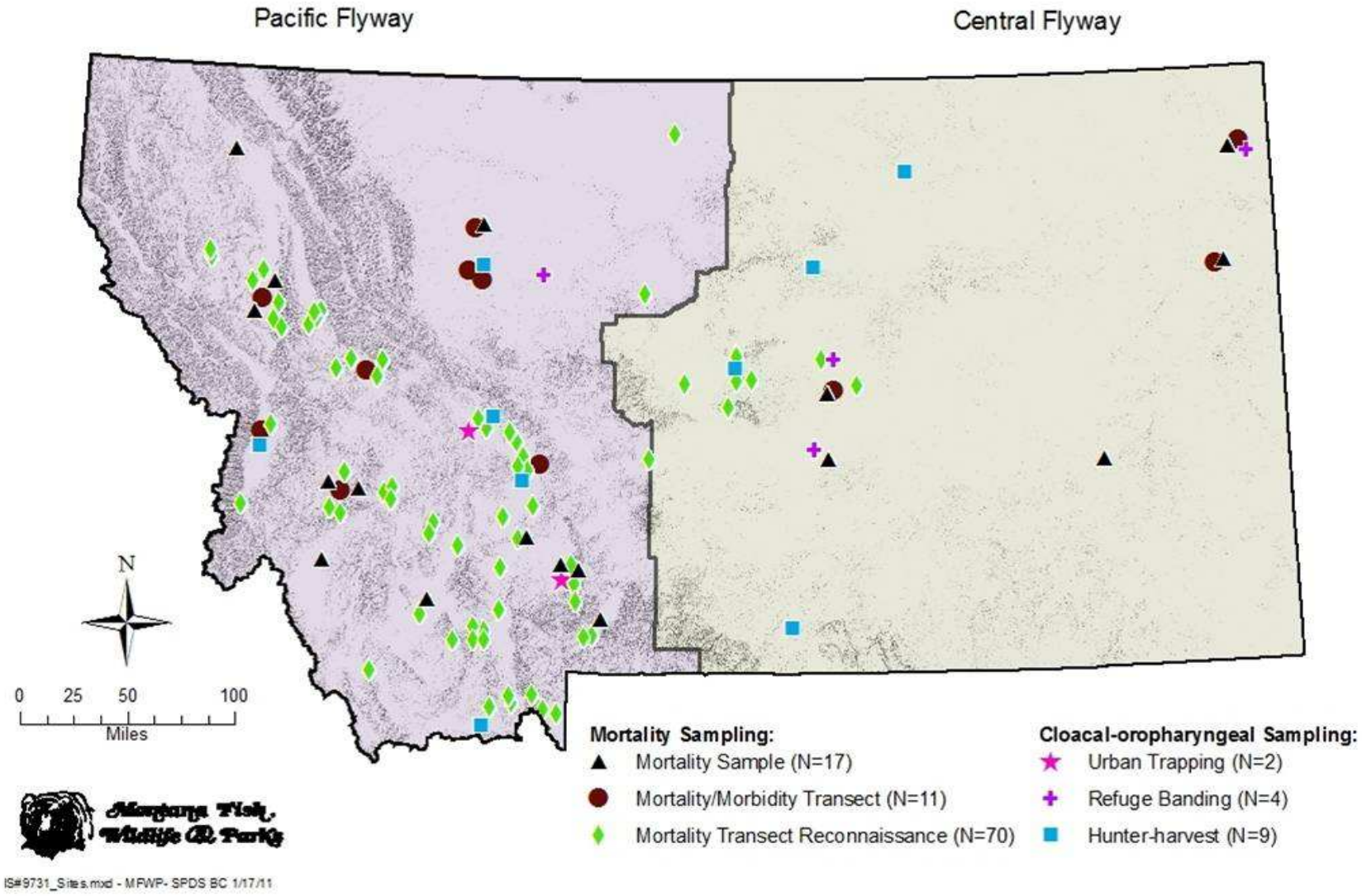

Figure 1. The Pacific and Central Flyways in Montana, and sampling sites for the 2010 Montana AI surveillance.

bird surveillance in Montana included those that tested positive for LPAI H5 or H7 in previous years of AI surveillance. The tundra swan, trumpeter swan, lesser snow goose, Ross's goose, and dabbling ducks were considered species of primary concern, as they have demonstrated the ability to asymptomatically shed HP-H5N1, as well as succumb to the disease (Brown *et al.* 2008, Kalthoff *et al.* 2008, Hars *et al.* 2008). These primary species move between Asia and North America and could contact HP-H5N1 directly from Asian bird populations (Alaska Interagency HPAI Bird Surveillance Working Group 2006). Diving ducks were considered secondary species from which samples should be collected. High numbers of most of these species migrate through Montana and provide opportunity for sampling via refuge trapping and banding operations, waterfowl hunting, and urban trapping (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2006). Hybrid semi-domestic geese and mallards at urban ponds served as sentinel species (Appendix 1).

Field Effort

Live bird AI sampling was conducted in conjunction with waterfowl banding at Benton Lake and Medicine Lake National Wildlife Refuges during August and September using methods approved by the U.S. Fish and Wildlife Service and Canadian Wildlife Service (1977). Net-launchers were used at three sites at Benton Lake and swim-in traps were employed at three sites at Medicine Lake. Trapping efforts were rotated between sites. Waterfowl were banded by USFWS biologists and cloacal and oropharyngeal samples were taken by AI personnel. Sampled birds were then released. Swim-in traps also were employed at Lake Mason and War Horse National Wildlife Refuges during July through September, though no banding was performed at these refuges during 2010.

Hunter-harvested waterfowl sampling began in late September and was conducted through November. Waterfowl were sampled at Bowdoin, Lee Metcalf, and Red Rocks Lakes National Wildlife Refuges, Freezeout Lakes and multiple sites on creeks and wetlands throughout the state. Hunter participation was voluntary and information about AI and the surveillance was distributed to hunters onsite. Sampling concluded when hunting diminished and as lakes froze.

Urban wild and semi-domestic bird sampling began in mid-October and ran through November concurrently with hunter-harvest sampling. AI personnel used swim-in traps at two urban ponds in central Montana to collect cloacal and oropharyngeal samples. The traps were modified for use on land at the Lewis and Clark Fairgrounds Pond in Helena and the MSU Pond in Bozeman. Permission to trap was granted by city and university managers, while FWP Information and Education personnel notified the public of trapping activities.

Laboratory Testing

Cloacal-oropharyngeal samples were submitted to the MDoL and tested using real-time reverse transcription-polymerase chain reaction (rRT-PCR). All samples were screened individually with a matrix gene primer/probe set designed to detect all influenza-A viruses. Samples testing positive were further analyzed to identify H5 and H7 subtypes (Spackman *et al.* 2002, Munster *et al.* 2009). Samples that screened positive or suspect for H5 or H7 were then sent to NVSL in Ames, Iowa, where confirmatory testing was performed for H5 and H7 subtypes using rRT-PCR and a standard rRT-PCR for N1. Virus isolation was also performed

by NVSL on all samples to confirm AI virus isolates and determine whether or not H5 and N1 were linked in the same viral strain. All samples that produced positive results using virus isolation were tested for pathogenicity using chicken inoculation studies and/or, if enough RNA was present in the clinical sample, a target amino acid sequence analysis was performed to determine virulence potential of the virus (U.S. Department of the Interior Fish and Wildlife Service 2006). LPAI prevalence was defined as the percentage of samples that tested positive for LPAI according to rRT-PCR results.

Sampling Effort

AI personnel collected 833 cloacal-oropharyngeal samples toward the sampling goal for Montana during 2010; 600 samples were collected by FWP and 233 by WS (Table 1). Refuge trapping operations yielded 331 samples and urban trapping efforts produced 104 samples for a total of 435 live bird samples (52%). Hunter-harvested samples totaled 398. Sampling effort consisted of 58 total sampling days; refuge trapping produced 18 sample days, urban trapping yielded 4, while hunter-harvest produced 36. Sampling effort across all swab sampling methods resulted in overall means of 3.9 sample days/site and 14.4 samples/sample day across 15 sites. Urban trapping yielded the highest mean number of samples/sampling day (26.0) among sampling methods, while hunter-harvest yielded the lowest (11.1).

Table 1. 2010 Montana AI surveillance swab sampling effort according to method.

	Sampling Method			Total
	Refuge trapping	Urban trapping	Hunter-harvest	
Number of samples	331	104	398	833
Percentage of total samples	40	12	48	100
Total sample days	18	4	36	58
Number of sites	4	2	9	15
Sample days/sample sites	4.5	2.0	4.0	3.9
Samples/sample day	18.4	26.0	11.1	14.4

The Montana sampling plan (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2006) called for high numbers of cloacal-oropharyngeal samples from primary species of concern and a focus on samples from secondary species to spread sampling effectively across species. Primary species comprised 91% (n=759) of the total samples collected. Samples from mallards (n=369) constituted 49% of the primary species and 44% of all cloacal-oropharyngeal samples. The remaining 51% of primary species samples collected were from taken from 10 species. Secondary species of concern comprised 9% (n=74) of the total cloacal-oropharyngeal samples obtained (Table 6).

Montana cloacal and oropharyngeal sampling effort was spread temporally throughout fall in conjunction with refuge trapping operations 7/20 – 9/23, during the harvest of waterfowl 9/25 – 11/25, and urban wild bird sampling 10/14 – 11/29. Sampling peaked on 10/2, opening day of general waterfowl hunting in Montana, and ended in late November as fall migration subsided. Primary species sampling began with mallards and gadwalls during refuge trapping in late July and northern pintails in September, while tundra and trumpeter swan, lesser snow

goose, and Ross's goose sampling occurred throughout October and November. Additional primary and secondary duck species were sampled quite consistently throughout the hunting season. Sentinel birds (hybrid geese and ducks) were sampled at urban ponds in October and November (Figure 2). Spatially, most cloacal-oropharyngeal samples from primary species were collected in the northeastern portion of the Montana Pacific Flyway. Samples collected from secondary species were distributed fairly evenly across Montana. As in previous years, Freezeout Lake was the most productive site, which yielded 26.3% (n=219) of the total swab samples collected, 119 of which were taken from primary species. Benton Lake yielded 176 samples (21%), all of which were collect from primary species of concern. Medicine Lake NWR in the northeast corner of the Montana Central Flyway yielded 13% of all samples (Figure 3).

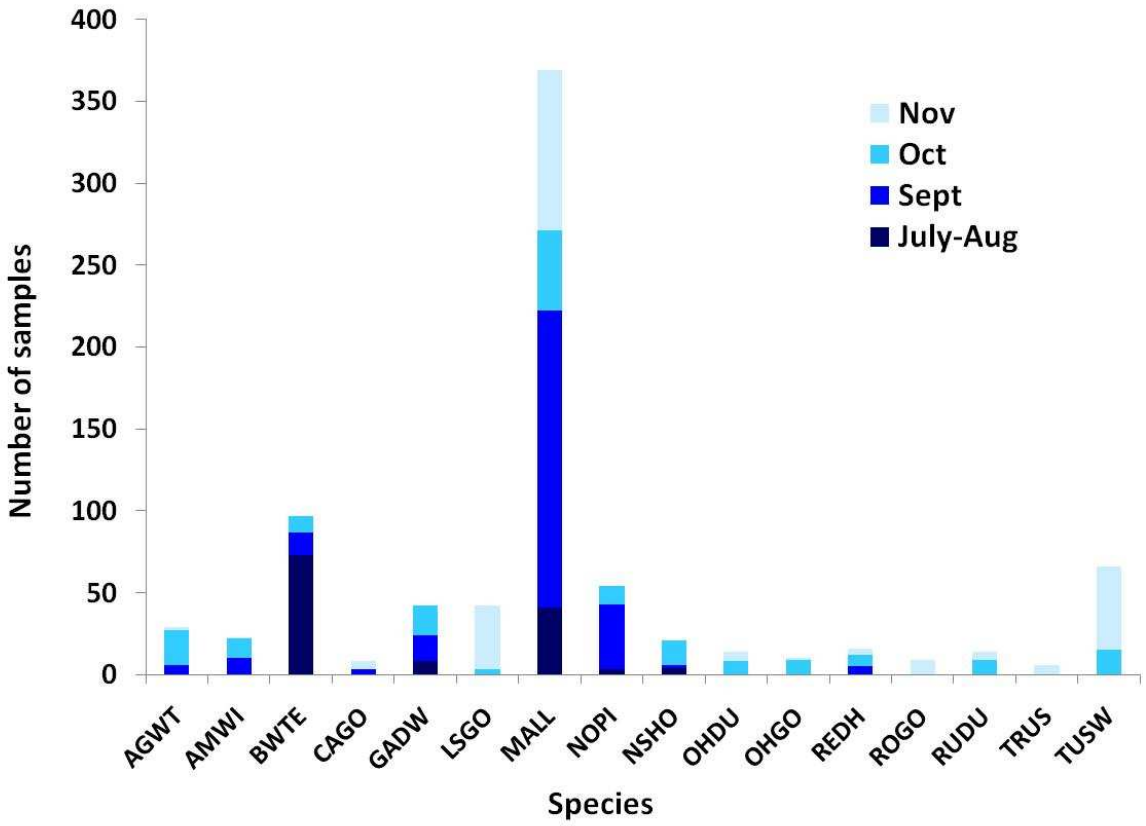

Figure 2. Temporal distribution of 2010 Montana AI cloacal and oropharyngeal sampling according to species. Species from which ≤3 samples were collected were excluded (lesser scaup: n=3; canvasback, common goldeneye, eared grebe: n=2 each; greater scaup, long-tailed duck, ringed-necked duck, wood duck: n=1 each).

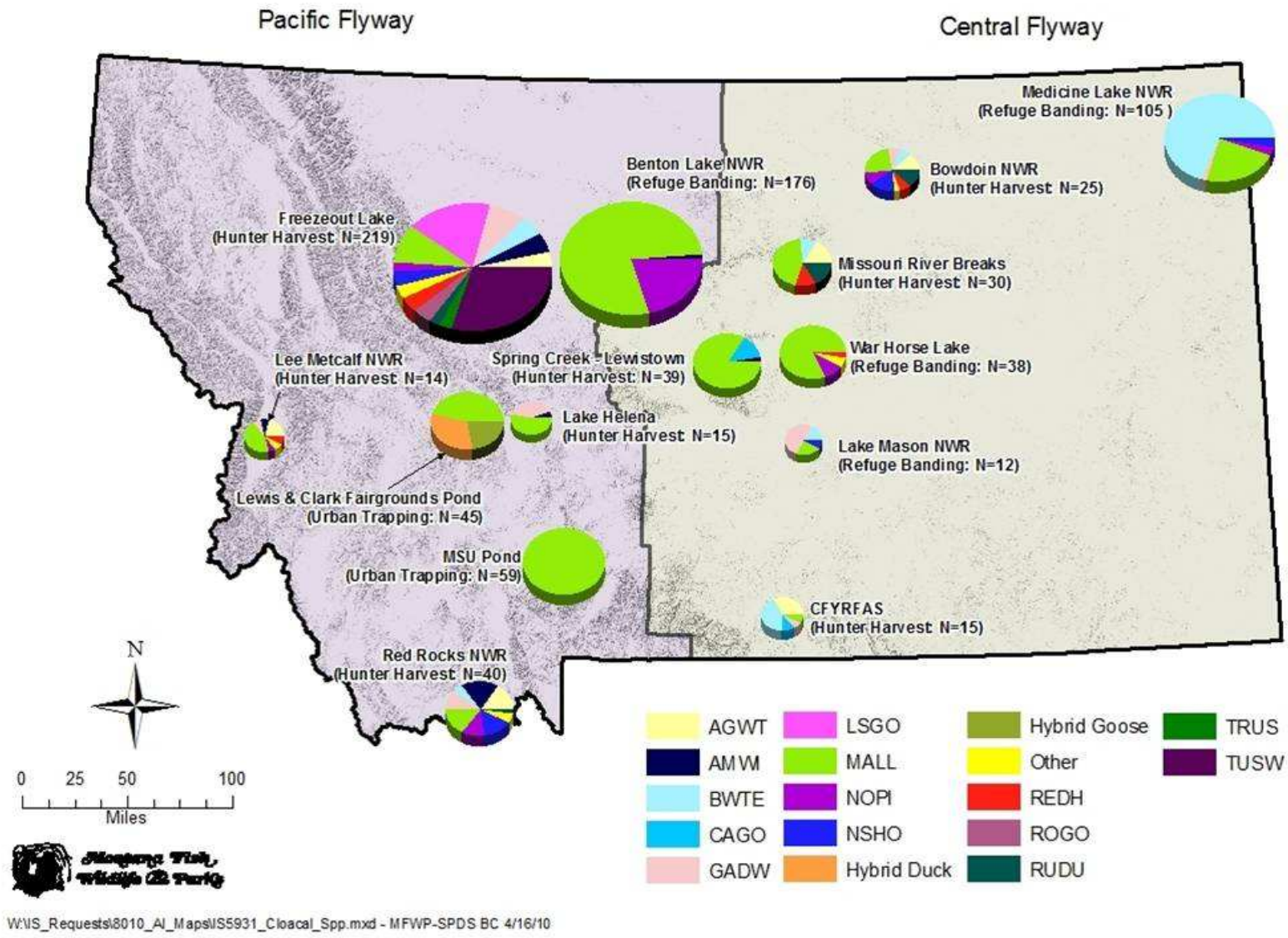

Figure 3. Spatial distribution of the 2010 Montana AI cloacal and oropharyngeal sampling according to species. The "Other" category combines all species from which ≤3 samples were collected (lesser scaup: n=3; canvasback, common goldeneye, eared grebe, n=2 each; greater scaup, ring-neck duck, wood duck: n=1 each). The long-tailed duck sample taken from the Missouri River was excluded from the map. Species codes are located in Appendix 1. Acronyms were used for the Clark Fork Yellowstone River Fishing Access Site (CFYRFAS) and National Wildlife Refuges (NWR).

Mortality/Morbidity Sampling

The Montana sampling plan supplement (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2007) specified the collection of ≤200 opportunistic mortality/morbidity samples within the AI sampling period. Reports made by the public were investigated according to the AI sampling criteria, which included consideration of the reported species as a potential concern for the presence of HP-H5N1 and the circumstances under which the dead or sick birds were found. Morbid birds were euthanized in accordance with the Guidelines for Euthanasia of Non-domestic Animals (AAZV 2006). Bird carcasses found within 24 hours of death with no sign of scavenging and euthanized birds were shipped for necropsy and disease testing at NWHC in Madison, WI.

Lab Testing

NWHC tested tracheal and cloacal swab samples and tissues by direct extraction. Testing procedures followed those described for cloacal-oropharyngeal sample testing. Samples that tested positive for either H5 or H7 were sent to NVSL for confirmation (Spackman *et al.* 2002, Munster *et al.* 2009).

Sampling Effort

A total of 21 FWP and USFWS mortality/morbidity samples were tested for AI by NWHC during the 2010 season. Carcasses from 15 species from 17 mortality events were collected statewide (Table 2). The 26 calls received by FWP about dead and dying birds yielded six mortality/morbidity sampling events while nine events were discovered while performing mortality/morbidity transects. The remaining samples were fielded by agency personnel. Of the 14 birds categorized by age and sex, five were classified as hatch-year birds (1 female, 1 male, 3 undetermined sex) and 9 were classified as after-hatch-year birds (6 females, 2 males, 1 undetermined sex).

Table 2. 2010 Montana AI mortality/morbidity samples tested for AI by NWHC according to species.

Species	Number of samples
American Coot	1
Avocet	1
Bald Eagle	2
Bufflehead	1
California Gull	1
Eurasian Collared-Dove	2
Great-horned Owl	2
Herring Gull	1
Mallard	1
Northern Harrier	1
Northern Raven	2
Red Crossbill	2
Trumpeter Swan	2
Willet	1
Wood Duck	1
Total	21

Mortality/Morbidity Transect Surveys

FWP AI personnel conducted weekly prospective transects to systematically survey species of concern throughout the state of Montana for morbidity and mortality (Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana 2007). Species identified as sensitive to highly pathogenic infection likely resulting in clinical disease and death were targeted for surveillance during fall migration until freeze-up (U.S. Department of the Interior Fish and Wildlife Service 2008). Priority species included tundra and trumpeter swans, American wigeon, canvasback, lesser scaup, northern shoveler, redhead, ring-necked duck, and wood duck, as well as shorebirds, grebes, terns, gulls and raptors (Becker 1966, Van Borm *et al.* 2005, Brown *et al.* 2006, Brown *et al.* 2007, Brown *et al.* 2008, Hall *et al.* 2009). Reconnaissance was conducted throughout the Montana Pacific and Central Flyways on lakes and wetlands in early and late fall to find sites for surveillance based on location, water conditions, access, and target species abundance. Once established, surveys were conducted every 5-9 days and evaluated based on the presence of priority species. Six sites across the state were concurrently surveyed and alternate locations were substituted when numbers of target species declined due to migration (Figure 1). Surveillance was terminated at a site when total target species numbered $\leq$200, a site was inaccessible due to winter conditions, or the lake or wetland froze over.

Transects contoured within ten feet of the shoreline to detect morbidity and mortality events either by canoeing or walking. To record the presence of target species and index abundance, censuses were conducted with spotting scopes and high-powered binoculars from a single point on each transect that allowed maximum visibility to the observer. To avoid double counting during the performance of individual surveys, only numbers of each species counted upon initial sighting were recorded to yield a minimum number, and only counts of additional target species not seen during the initial census were added during the survey. Because it is likely bird populations were recounted across consecutive surveys, census data were reported as "bird observations". All symptomatic or dead birds of suitable quality were collected and tested for AI by submission of intact carcasses to NWHC following the protocols described above.

Sampling Effort

Eleven mortality/morbidity transects were established and conducted between 6/9 and 11/24 that yielded a total of 200 weekly surveys. Transect routes ranged from 2 to 9 km in length and averaged 4.27 ($\pm$2.15) km. Completed surveys ranged from 5 to 240 minutes and averaged 125 ($\pm$46.84) minutes for a total of 417 hours. An additional 108 reconnaissance surveys on 70 lakes and wetlands across the state began 6/14 and ended 11/3 (Table 3). A total of 114,149 bird observations were recorded upon initial sighting of target species during the 2010 surveys, over half of which were ducks, geese, and swans. Nearly one third of the birds observed were gulls and terns, about one tenth were grebes, and most of the remaining tenth was comprised of shorebirds and raptors (Table 4). Dead and sick birds found on transects totaled 67 and 15, respectively. The carcasses identifiable to species were comprised of 35 California gulls, four American white pelicans and American coots, three horned grebes and western grebes, two herring gulls and green-winged teal, and single carcasses from 12 additional species. Twelve of the carcasses collected on transects were sent to NWHC to test for AI and determine cause of death.

Table 3. 2010 Montana AI mortality/morbidity transect survey start and end dates, length and average survey times for complete surveys.

Transect	Date		Transect	Average (total)	Number
	start	end	length (km)	survey time (min)	of surveys
Brown's Lake	8/14	10/12	9	176	15
Canyon Ferry, Pond 2	6/10	11/24	6	139	25
Eyraud Lakes	6/15	11/12	5	88	22
Fox Lake	6/14	11/15	2	96	23
Freezeout Lake, Pond 6	10/08	11/12	3	99	5
Freezeout Lake, NW Bay	6/15	10/20	4	84	17
Georgetown Lake	6/14	11/22	4	116	24
Lee Metcalf, Pond 8	10/27	11/09	2	92	3
Medicine Lake, Sayer Bay	6/15	11/15	4	136	23
Ninepipes Reservoir	7/15	11/08	6	203	18
Yellow Water Reservoir	6/09	11/22	2	121	25
Total	6/09	11/24	47	125 (24,995)	200
Transect reconnaissance	6/14	11/03	---	49 (5,420)	108

Table 4. Montana 2010 mortality/morbidity transect survey bird observations according to family.

Family	Number counted (%)	
Anatidae (duck, goose, swan)	65,068	(57)
Laridae (gull, tern)	33,297	(29)
Podicipedidae (grebe)	11,463	(10)
Scolopacidae (sandpiper, phalarope)*	2,221	(2)
Charadriidae (plover, killdeer)	1,099	(1)
Recurvirostridae (avocet, stilt)	623	(>1)
Accipitridae/Falconidae/Strigidae (raptors)	378	(>1)
Total	114,149	(100)

*Includes curlew, dowitcher, godwit, sanderling, sandhill crane, snipe, willet, yellowlegs, unidentified shorebirds.

Data Management, Reporting of Results, Statistics

AI personnel entered cloacal and oropharyngeal sampling data directly into the NVSL national web-based database system. NVSL reported all cloacal-oropharyngeal sample results through the same database, which included H5, H7, and N1 screening results, as well as LPAI subtype and pathogenicity. Montana 2010 cloacal and oropharyngeal data and results were uploaded to FWP's existing AI database. Results were then used to calculate confidence intervals for the proportion of LPAI positive cloacal-oropharyngeal swab samples according to species (R Core Development Team, 2006). Using the Agresti-Coull interval, the assumptions were 1) sampling was random or at least representative of the entire population, 2) LPAI rates were the same temporally, spatially and across trapping methods, and 3) there was no measurement error. Confidence intervals for sex and age classes of individual species were not calculated due to the large differences in the proportion of LPAI positive samples within each sex and age class. The outcomes of AI and additional disease testing, as well as cause of death when possible, were reported directly to FWP by NWHC. AI mortality/morbidity transect survey data and NWHC results were entered into FWP databases.

RESULTS

While AI virus was found in samples, HP-H5N1 was not detected in Montana during the 2010 surveillance. Because the AI surveillance did not focus on the detection of LPAI, samples that tested LPAI positive but H5 and H7 negative were not tested with virus isolation to determine AI subtype.

Cloacal-oropharyngeal Samples

LPAI Results

Of the total 833 cloacal-oropharyngeal samples, 251 (30%) tested positive for LPAI, and refuge trapping yielded the highest LPAI prevalence within sample collection method (57%: Table 5).

Table 5. 2010 Montana LPAI positive cloacal-oropharyngeal sample numbers and prevalence according to method of sample collection.

Sampling method	Number of samples	Number of LPAI positive samples	LPAI prevalence within method (%)
Hunter-harvest	398	52	13
Refuge trapping	331	194	57
Urban trapping	104	5	5
Total	833	251	30

According to temporal analysis, the peak for LPAI prevalence across sex and age classes was in September, excluding hatch-year females (Figure 4). LPAI prevalence increased from August to September and then declined across the rest of the sampling season, with the exception of hatch-year females which decreased throughout the fall.

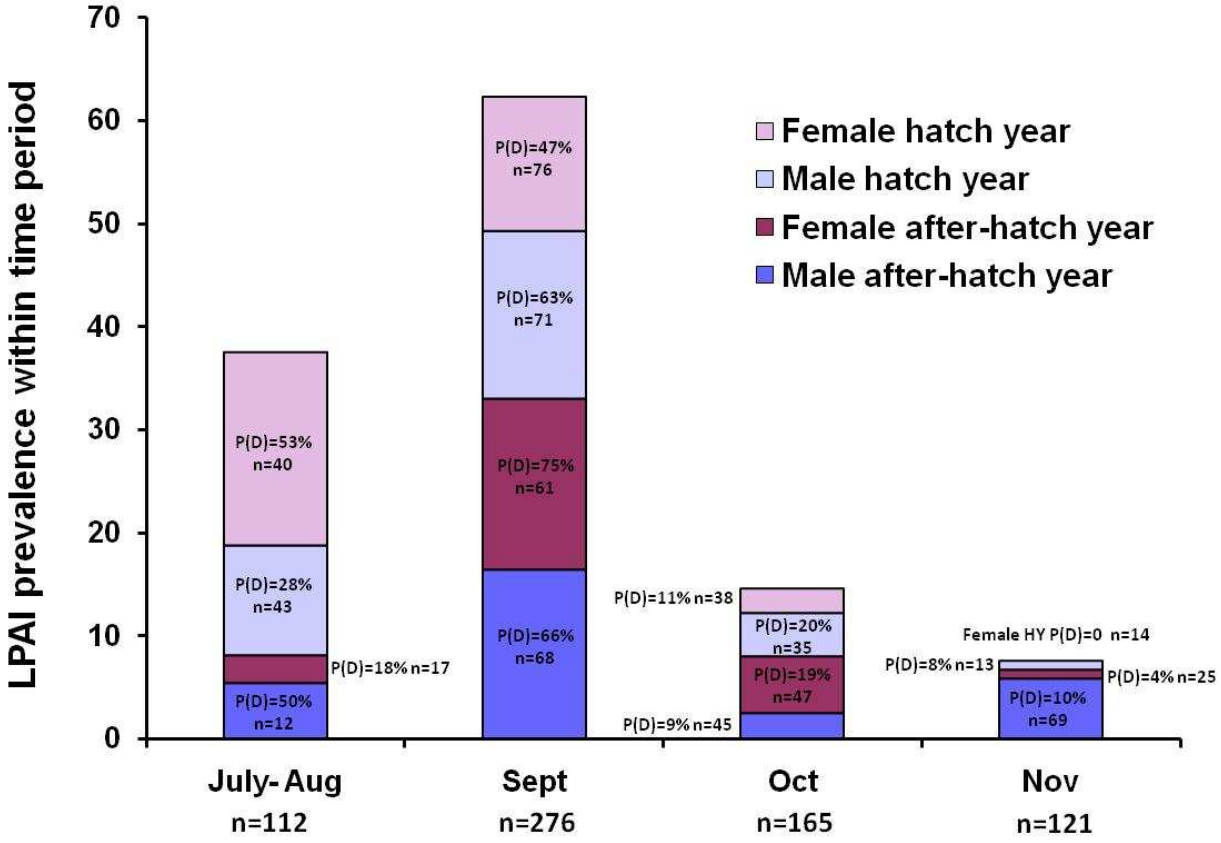

Figure 4. 2010 LPAI prevalence of cloacal-oropharyngeal samples by time period. Only birds for which age and sex were determined were included (n=674). Prevalence according to each sex and age class within time period is indicated within graph bars; P(D) = prevalence of disease.

Known sex and age classes across all sampled species and methods were pooled for species-specific analysis which resulted in an average LPAI prevalence of 30%. Northern pintail had the highest LPAI prevalence (54%) within the primary species of concern, while LPAI prevalences for mallard and blue-winged teal were 45% and 35%, respectively. LPAI prevalences were 24% or less for the remaining eight primary species and all secondary species of concern (Table 6).

Table 6. Proportion of 2010 Montana cloacal-oropharyngeal swab samples to test LPAI positive according to species using the Agresti-Coull interval. Mean= proportion of LPAI positive samples within species, Lower CI= lower 95% Confidence Interval, Upper CI= upper 95% Confidence Interval, X= number of LPAI positive samples within species, N= number of birds sampled within species. The "Other" category is comprised of species from which ≤3 samples were collected (lesser scaup: n=3; canvasback, common goldeneye, eared grebe: n=2 each; greater scaup, long-tailed duck, ringed-necked duck, wood duck: n=1 each).

	Species (n=24)	Mean	Lower CI	Upper CI	X	N
Primary species	Northern Pintail	0.54	0.41	0.66	29	54
	Mallard	0.45	0.40	0.50	166	369
	Blue-winged Teal	0.35	0.26	0.45	34	97
	Northern Shoveler	0.24	0.10	0.45	5	21
	American Green-winged Teal	0.21	0.09	0.39	6	29
	American Wigeon	0.13	0.04	0.33	3	23
	Ross's Goose	0.11	0.00	0.46	1	9
	Gadwall	0.07	0.02	0.20	3	42
	Tundra Swan	0.03	0.00	0.11	2	66
	Lesser Snow Goose	0.00	0.00	0.10	0	42
	Trumpeter Swan	0.00	0.00	0.44	0	6
Secondary species	Redhead	0.13	0.02	0.37	2	16
	Ruddy Duck	0.00	0.00	0.25	0	14
	Hybrid Duck	0.00	0.00	0.25	0	14
	Hybrid Goose	0.13	0.00	0.32	0	10
	Canada Goose	0.00	0.00	0.37	2	8
	Other (8 species)	0.00	0.00	0.27	0	13
	Total	0.30	0.27	0.33	251	833

H5, H7, and N1 Results

Fourteen cloacal-oropharyngeal samples tested positive for H5 during 2010, of which 13 produced N1 negative results. One female adult mallard from the Missouri River Breaks tested positive for H5 and N1 using RRT-PCR and VI. The H5N1 virus was classified as low pathogenic using target amino acid sequence analysis. H7 was not detected in the 2010 Montana samples.

Mortality/Morbidity Samples

Of the 21 mortality/morbidity samples submitted for examination to NWHC, three American coots produced presumptive LPAI positive results and negative results for H5, H7, and N1. Cause of death for mortality events were reported to individual submitters by FWP and were not included in this report.

DISCUSSION

AI virus in low pathogenic form was detected in Montana samples during the 2010 sampling season as expected, while HP-H5N1 has not been found to date in Montana or elsewhere in North America. Twelve birds tested H5 positive and N1 negative across all sampling methods, while one bird tested positive for LPAI H5N1. When all data were pooled, 30% of the samples tested positive for LPAI.

Hunter-harvest swab sampling produced the most samples (48%) across all methods and a LPAI prevalence of 13% within method, while refuge trapping yielded 40% of the total swab samples and the highest LPAI prevalence (57%) within method. The highest proportion of LPAI positive samples occurred in September (62%) and then declined throughout fall, which was consistent among all years of AI surveillance in Montana. Timing of refuge trapping verses hunter-harvest and urban trapping sampling may partially explain this difference. Several studies have shown that AI is more prevalent in early fall and decreases as fall migration proceeds (Stallknecht 2003, Gilbert *et al.* 2006). Changes in LPAI prevalence may be due to a combination of pre-migration waterfowl density and the high number of immunologically naïve juveniles in early fall. Subsequent declines in LPAI may be a result of increased flock immunity and progressive dispersal of bird populations (Stallknecht 2003, Gilbert *et al.* 2006). The use of different trapping methods may also contribute to the differing low pathogenic AI results.

Northern pintails produced the highest LPAI prevalence (54%) among all species tested during 2010. Recent studies have shown that northern pintails carry numerous strains of LPAI with some of the highest prevalences among water bird species (Hinshaw *et al.* 1980, Runstadler *et al.* 2007, Ip *et al.* 2008, Parmley *et al.* 2008). Hatch-year northern pintails tested in Alaska produced higher prevalences than the adults, while hatch-year males and females differed little (Ip *et al.* 2008). Similar results were apparent in Montana. The Montana 2010 northern pintail LPAI prevalence for adult (after hatch-year) males and females differed little, 40% (n=5) and 38% (n=13), respectively, and were lower than the prevalence for hatch year northern pintails. However, the 70% hatch year male northern pintail (n=14) LPAI prevalence was highest among the sex and age classes and differed from hatch year female (n= 22) LPAI prevalence, which was 50%.

Success of wild live and hunter-harvested bird sampling, as well as mortality/morbidity sampling, depends on the availability of the species and numbers of birds during migration. The timing of migration can be affected by many factors, including climate and weather patterns (Blokpoel and Richardson 1978, Nichols *et al.* 1983, Harmata *et al.* 2000), age of the migrants (Hepp and Hines 1991), population size (Nichols *et al.* 1983), and bird body mass, especially in hatch-year birds (Owen and Black 1989). It was important to obtain high numbers of hatch-year bird samples because that age class likely contained the highest prevalence of AI viruses during their first fall migration (Olsen *et al.* 2006); this was accomplished during each year of Montana AI surveillance. Though mallards were the most abundant and available waterfowl in Montana, mallard sampling was limited to maximize sampling of other target species. Urban trapping provided the greatest temporal flexibility among swab sampling methods, as sampling could be conducted according to schedule rather than opportunistically, but afforded the least diversity of species (n=3). Conversely, hunter-harvest sampling was difficult to allocate temporally while it provided the most species diversity (n=24); 11% of the total hunter-harvest samples were

collected during the first weekend of the waterfowl hunting. Refuge trapping provided seven species and took place early in the sampling period at four National Wildlife Refuges. To distribute sample collection temporally across species during the 2010 surveillance, emphasis was placed on sampling northern pintails during refuge trapping in early fall, and tundra swans and lesser snow geese during hunter-harvest later in fall when these species were most available. Wild sentinel birds at urban ponds were sampled concurrently with hunter-harvest birds.

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LITERATURE CITED

Al-Azemi A, J Bahl, S Al-Zenki, Y Al-Shayji, S Al-Amad, H Chen, Y Guan, JSM Peiris, and GJD Smith. 2008. Avian influenza A virus (H5N1) Outbreaks, Kuwait, 2007. Emerging Infectious Diseases 14: 958-961.

Alaska Interagency HPAI Bird Surveillance Working Group. 2006. Sampling protocol for highly pathogenic Asian H5N1 avian influenza in migratory birds in Alaska. Interagency planning report, Anchorage, AK.

American Association of Zoo Veterinarians (AAZV). 2006. Guidelines for euthanasia of non-domestic animals. AAZV, USA.

Becker WB. 1966. The isolation and classification of Tern virus: Influenza virus A-Tern South Africa, 1961. The Journal of Hygiene 64: 309-320.

Blokpoel H and WJ Richardson. 1978. Weather and spring migration of Snow Geese across southern Manitoba. Oikos 30: 350-363.

Brown JD, DE Stallknecht, and DE Swayne. 2008. Experimental infection of swans and geese with highly pathogenic avian influenza virus (H5N1) of Asian lineage. Emerging Infectious Diseases 14: 136-142.

Brown JD, DE Stallknecht, Valeika S and DE Swayne. 2007. Susceptibility of wood ducks to H5N1 highly pathogenic avian influenza virus. Journal of Wildlife Diseases 43: 660-667.

Brown JD, DE Stallknecht, JR Beck, DL Suarez, and DE Swayne. 2006. Susceptibility of North American ducks and gulls to H5N1 highly pathogenic avian influenza viruses. Emerging Infectious Diseases 12: 1663-1670.

Central Flyway Council. 2006. Surveillance plan for early detection of highly pathogenic avian influenza-H5N1 in migratory birds in the Central Flyway. Unpublished report, April 2006.

Chen H, KS Li, J Wang, XH Fan, JM Rayner, D Vijaykrishna, JX Zhang, LJ Zhang, CT Guo, CL Cheung, KM Xu, L Duan, K Huang, K Qin, YHC Leung, WL Wu, HR Lu, Y Chen, NS Xia, TSP Naipospos, KY Yuen, SS Hassan, S Bahri, TD Nguyen, RG Webster, JSM Peiris, and Y Guan. 2006. Establishment of multiple sublineages of H5N1 influenza virus in Asia: implications for pandemic control. Proceedings of the National Academy of Sciences 103: 2845-2850.

Chen R and EC Holmes. 2009. Frequent inter-species transmission and geographic subdivision in avian influenza viruses from wild birds. Virology 383, 156-161.

Dugan VG, R Chen, DJ Spiro, N Sengamalay, J Zaborsky, E Ghedin, J Nolting, DE Swayne, JA Runstadler, GM Happ, DA Senne, R Wang, RD Slemons, EC Holmes, and JK Taubenberger. 2008. The evolutionary genetics and emergence of avian influenza viruses in wild birds. PLoS Pathogens 4: e1000076.

Gilbert M, X Xiao, J Domenech, J Lubroth, V Martin, and J Slingenbergh. 2006. Anatidea migration in the Western Palearctic and spread of highly pathogenic avian influenza H5N1 virus. Emerging Infectious Diseases 12: 1650-1656.

Hall JS, HS Ip, JC Franson, C Meteyer, S Nashold, JL TeSlaa, J French, P Redig, and C Brand. 2009. Experimental infection of a North American raptor, American kestrel (falco sparverius) with highly pathogentic avian influenza virus (H5N1). PLoS ONE 4:e7555.

Harmata AR, KM Podruzny, JR Zelenak, and ML Morrison. 2000. Passage rates and timing of bird migration in Montana. The American Midland Naturalist 143: 30-40.

Hars J, S Ruette, M Benmergui, C Fouque, J Fournier, A Legouge, M Cherbonnel, B Daniel, C Dupuy, and V Jestin. 2008. The epidemiology of highly pathogenic H5N1 avian influenza in mute swans (*Cygnus olor*) and other Anatidae in the Dombes Region (France), 2006. Journal of Wildlife Diseases 44: 811-823.

Hepp GR and JE Hines. 1991. Factors affecting winter distribution and migration distance of wood ducks from southern breeding populations. Condor 93: 884-891.

Hinshaw VS, RG Webster, and B Turner. 1980. The perpetuation of orthomyxoviruses and paramyxoviruses in Canadian waterfowl. Canadian Journal of Microbiology 26: 622-629.

Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana. 2006. Surveillance for early detection of highly pathogenic avian influenza H5N1 in wild birds: 2006 Montana sampling plan. Unpublished report, May 2006.

Interagency Coordinating Committee for HPAI H5N1 Wild Bird Surveillance in Montana. 2007. Surveillance for early detection of highly pathogenic avian influenza H5N1 in wild birds: Montana sampling plan supplement for 2007. Unpublished report, June 2007.

Interagency Asian HPAI Early Detection Working Group. 2006. An early detection system for Asian H5N1 highly pathogenic avian influenza in wild migratory birds: U.S. interagency strategic plan. Unpublished report, December 2005.

Ip HS, PL Flint, JC Franson, RJ Dusek, DV Derkson, RE Gill, CR Ely, JM Pierce, RB Lanctot, SM Matsuoka, DB Irons, JB Fischer, RM Oates, MR Peterson, TF Fondell, DA Rocque, JC Peterson, and TC Rothe. 2008. Prevalence of Influenza A viruses in wild migratory birds in Alaska: patterns of variation in detection at a crossroads of intercontinental flyways. Virology Journal 5: 71.

Kalthoff D, A Breithaupt, JP Teifke, A Globig, T Harder, TC Mettenleiter, and M Beer. 2008. Highly pathogenic avian influenza virus (H5N1) in experimentally infected adult mute swans. Emerging Infectious Diseases 14: 1267-1270.

Krauss S, CA Obert, J Franks, D Walker, K Jones, P Seiler, L Niles, SP Pryor, JC Obenauer, CW Naeve, L Widjaja, RJ Webby, and RG Webster. 2007. Influenza in migratory birds and evidence of limited intercontinental virus exchange. PLoS Pathogens 3: e167.

Lvov DK, MIU Schelkanov, PG Deriabin, TV Grebennikova, AG Prilipov, YA Nepoklonov, GG Onishchenko, NA Vlasov, TI Aliper, AD Zaberezhnyi, YD Kireyev, OP Krasheninnikov, ST Kiryukhin, YI Burtseva, and AN Slepushkin. 2006. Isolation of Influenza A/H5N1 virus strains from poultry and wild birds in west Siberia during epizooty (July 2005) and their depositing to the state collection of viruses (August 2005). Problems of Virology (Voprosy Virusology) 51: 11-14.

Munster VJ, C Baas, P Lexmond, TM Bestebroer, J Guldemeester, WEP Beyer, E de Wit, M Schutten, GF Rimmelzwaan, ADME Osterhaus, and RAM Fouchier. 2009. Practical considerations for high-throughput Influenza A virus surveillance studies of wild birds by use of molecular diagnostic tests. Journal of Clinical Microbiology 47: 666-673.

Nichols JD, KJ Reineck, and JE Hines. 1983. Factors affecting the distribution of mallards wintering in the Mississippi. Auk 100: 932-946.

Olsen B, VJ Munster, A Wallensten, J Waldenstrom, ADME Osterhaus, and RAM Fouchier. 2006. Global patterns of Influenza A virus in wild birds. Science 312: 384-388.

Owen M and JM Black. 1989. Factors affecting the survival of barnacle geese on migration from the breeding grounds. Journal of Animal Ecology 58: 603-617.

Pacific Flyway Council. 2006. Surveillance for early detection of highly pathogenic avian influenza H5N1 in wild migratory birds: a strategy for the Pacific Flyway. Unpublished report, March 2006.

Parmley EJ, N Bastien, TF Booth, V Bowes, PA Buck, A Breault, D Caswell, P Daoust, JC Davies, SM Elahi, M Fortin, F Kibenge, R King, Y Li, N North, D Ojkic, J Pasick, SP Pryor, J Robinson, J Rodrique, H Whitney, P Zimmer, and FA Leighton. 2008. Wild bird influenza survey, 2005. Emerging Infectious Diseases 14: 84-87.

Peterson AT, BW Benz, and M Papes. 2007. Highly pathogenic H5N1 avian influenza: entry pathways in North America via bird migration. PLoS ONE 2:e261.

R Core Development Team. 2006. R 2.4.1-a language and environment. R Foundation for Statistical Computing, Vienna, Austria. Available from http://www.R-project.org. Accessed 1 Sept 2007.

Runstadler JA, GM Happ, and RD Slemons. 2007. Using RT-PCR analysis and virus isolation to determine the prevalence of avian influenza infections in ducks at Minto Flats State Game Refuge, Alaska, during August 2005. Archives of Virology 152: 1901-1910.

Scholtissek C, W Rohde, V Von Hoyningen, and R Rott. 1978. On the origin of the human influenza virus subtypes H2N2 and H3N2. Virology 87: 13-20.

Spackman E, DA Senne, TJ Myers, LL Bulaga, LP Garber, ML Perdue, K Lohman, LT Daum, and DL Suarez. 2002. Development of a real-time reverse transcriptase PCR assay for type A influenza virus and the avian H5 and H7 hemagglutinin subtypes. Journal of Clinical Microbiology 40: 3256-3260.

Stallknecht DE. 2003. Ecology and epidemiology of avian influenza viruses in wild bird populations: waterfowl, shorebirds, pelicans, cormorants, etc. *In* Special Issue, Fourth International Symposium in Avian Influenza, 1997 Proceedings. Avian Diseases 47: 61-69.

Ungchusak K, P Auewarakul, SF Dowell, R Kitphati, W Auwanit, P Puthavathana, M Uiprasertkul, K Boonnak, C Pittayawonganon, NJ Cox, SR Zaki, P Thawatsupha, M Chittaganpitch, R Khontong, JM Simmerman, and S Chunstthiwat. 2005. Probable person-to-person transmission of avian influenza A (H5N1). New England Journal of Medicine 352: 333-340.

U.S. Department of the Interior Fish and Wildlife Service. 2006. Early detection and response plan for occurrence of highly pathogenic avian influenza in wild birds. Planning report, December 2006.

U.S. Department of the Interior Fish and Wildlife Service. 2008. Central Flyway highly pathogenic avian influenza H5N1: 2007-2008 surveillance annual report. Annual report, May 2008.

U.S. Fish and Wildlife Service and Canadian Wildlife Service. 1977. North American bird banding manual, Volume II. Bird banding techniques. U.S. Fish and Wildlife Service, Washington, D.C., USA, and Canadian Wildlife Service, Ottawa, Ontario, Canada.

USDA-APHIS-Wildlife Services, U.S. States and Tribes. 2010. Implementation plan for HPAI surveillance in wild migratory birds in the United States. Planning report, April 2010.

Van Borm S, I Thomas, G Hanquet, B Lambrecht, M Boschmans, G Dupont, M Decaestecker, and R Snacken. 2005. Highly pathogenic H5N1 influenza virus in smuggled Thai eagles, Belgium. Emerging Infectious Diseases 11: 702-705.

van Gils JA, VJ Munster, R Radersma, D Liefhebber, RAM Fouchier, and M Klaassen. 2007. Hampered foraging and migratory performance in swans infected with low-pathogenic avian influenza A virus. PLoS ONE 1:e184.

Weber TP and NI Stilianakis. 2007. Ecological immunology of avian influenza (H5N1) in migratory birds. Emerging Infectious Diseases 13: 1139-1143.

World Health Organization. 2007. Avian influenza fact sheet. Available from http://www.who.int/mediacentre/factsheets/avian_influenza/en/. Accessed 14 Mar 2007.

APPENDIX I

Target Species and Species Codes for Cloacal-oropharyngeal Sampling

	Species (n=24)	Species Code
Primary species	American Green-winged Teal	AGWT
	American Wigeon	AMWI
	Blue-winged Teal	BWTE
	Gadwall	GADW
	Lesser Snow Goose	LSGO
	Mallard	MALL
	Northern Pintail	NOPI
	Northern Shoveler	NSHO
	Ross's Goose	ROGO
	Trumpeter Swan	TRSW
	Tundra Swan	TUSW
	Wood Duck	WODU
Secondary species	Canada Goose	CAGO
	Canvasback	CANV
	Common Goldeneye	COGO
	Eared Grebe	EAGR
	Greater Scaup	GRSC
	Hybrid Duck	OHDO
	Hybrid Goose	OHGO
	Lesser Scaup	LESC
	Long-tailed Duck	LTDU
	Redhead	REDH
	Ring-necked Duck	RNDU
	Ruddy Duck	RUDU